Indian Journal of Geo Marine Sciences
Vol.46 (11), November 2017, pp. 2165-2174

Review Article

Microbial organic acids production, biosynthetic mechanism and applications -Mini review

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Received 30 June 2015 ; revised 08 April 2016

Organic acid constitute a significant portion of the fermentation market in the world, and microbial production is an important economic alternative to chemical synthesis for many of them. Thus, in order to address the growing market demands of organic acids with the passage of time, it needs to develop new strategies or discoveries for new or novel microbial strains for high level production of commercially important organic acid such as; gluconate, malate, and citrate. In present review, through cumulative analysis of the current microbial strains and their biosynthetic mechanisms for production of these acids, we present guidelines for future developments in this fast-moving field.

[Keywords: Alpha-ketoglutaric acid, Citric acid, Fumaric acid, Gluconic acid, Itaconic acid, Malic acid, and Biosynthetic mechanisms]

Introduction

An incentive of the environment to replace traditional chemical techniques with the biologically based production of organic acid has exposed fungi as actual striking cell factories. Fungi are particularly interesting industrial units for the production of less molecular weight organic compounds present in all organisms and having one or more carboxyl groups.[1]

Organic acids have attracted considerable attention for their role in natural ecology and their potential industrial applications as food additives, pharmaceutical and cosmetic excipients.[1] They are fully degradable molecules and can be used as chemical intermediates or as synthons for the production of biodegradable polymers, potentially replacing petroleum-based or synthetic chemicals. Some fungi are well recognized to produce high amounts of various useful organic acids for their natural capability.[2-3] Fungal natural production of organic acids is thought to have many key roles in nature depending on the species of fungi producing them.

This literature concerns about microbial organic acid production through fermentation in the large quantity or which compromises prospective for future developments.

Alpha-ketoglutaric acid

Alpha-ketoglutaric acid (KGA) is an important intermediate of the tricarboxylic acid (TCA) cycle and a major contributor to amino acid and protein metabolism. It has wide applications in the agrochemical and pharmaceutical industries. These applications produce an expansive market demand for KGA production.[4]

Alpha-ketoglutaric acid can be synthesized chemically through many steps from oxalic acid and succinic acid diethyl esters.[5] Many disadvantages of this method are concerned to the use of toxic chemicals in its production e.g., cyanides, presence of copper containing catalyst, generation of toxic waste and a decrease in product synthesis due to different by-products like other organic acids and glycine.[6]

The Microbial production of α-KGA by fermentation is under research from numerous decades.[7] Most of bacterial species including; *Arthrobacter paraffineus*, *Bacillus natto*, *Bacillus*

megatherium, *Bacillus mesentericus*, *Pseudomonas fluorescens*, *Bacterium succinicum* and *Corynebacterium glutamicum* and yeasts species such as; *Candida rugosa*, *Torulopsis glabrata*, *Candida catenulate*, *Pichia dispora*, *Pichia besseyi* and *Yarrowia lipolytica* have been used in different era for α-KG production ranging from 17±2.0 g/l to 185±3.1 g/l.[8]

An important multifunctional organic acid i.e. alpha-ketogluteric acid (α-KGA) is formed in the TCA cycle and holds an important position in amino acid metabolism.[5] The systematic scheme for production of α-KGA during TCA cycle involving different enzymes demonstrated in Figure 1. In which Pyruvate is converted into acetyl-CoA, which in turn enters into TCA cycle and leaded toward the production of KGA with the help of different enzymes mentioned below. Different studies exposed the quantities and percentages of organic acids produced in *Y. lipolytica* that can be affected through the enzyme activities changes in TCA.[9]

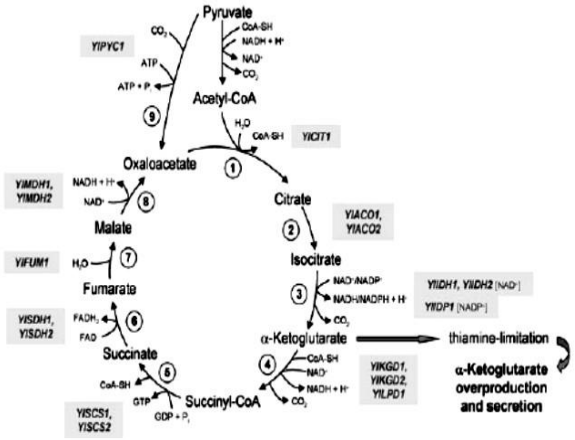

Figure 1- The tricarboxylic acid cycle in *Y. lipolytica* and the replacement of oxaloacetate through addition of carbon dioxide to pyruvate. In *Y. lipolytica* the eight cycle enzymes including anaplerotic enzyme, pyruvate carboxylase and the isoenzymes are encoded by following nuclear genes: YlACO1, YlIDH1, YlACO2 aconitase, YlIDH2 NAD+-dependent isocitrate dehydrogenase complex, YlCIT1 citrate synthase, YlIDP1 NADP+- dependent isocitrate dehydrogenase, YlSCS2 succinyl-coA-synthetase complex, YlKGD1, YlKGD2, YlSCS1, YlLPD1 α-ketoglutarate dehydrogenase complex, YlSDH1, YlSDH2 catalytic subunits of succinate dehydrogenase, YlMDH2 malate dehydrogenase, YlFUM1 fumarase, YlMDH1, YlPYC1 pyruvate carboxylase.[6]

The α-KGA possesses an extensive scope of applications, e.g. as a component of infusion solutions, as basic chemical unit for the heterocycles chemical synthesis, wound healing components and as a dietary supplement. Barrett and Yousaf[10] defined the thermal polycondensation of α-KGA to one of the triols glycerol, 1,2,6 hexanetriol or 1,2,4-butanetriol and resulting poly (triol-α-ketoglutarate) with extensive mechanical and chemical properties aselastomers, which has current possible applications in biomedicine, like drug delivery or tissue engineering. It is also used as a preliminary material for the formation of the novel pharmaceutical products possessing antitumor activity[5] and as an agent which improves athletic performance. It is also employed in biomedical studies as a substrate for measurement of α-ketoglutarate dehydrogenase, aspartate aminotransferase and alanine aminotransferase activities for the diagnosis of wide number of diseases such as; acute myocardial infarction, muscular dystrophy and hepatitis etc. The α-KGA is one of the most important nitrogen transporters in metabolic pathways.[11] It plays a role in detoxification of ammonia in brain, which in turn prevent Reye's syndrome, cirrhosis and urea cycle disorder. Furthermore, according to recent study α-KGA also play important role in significantly increasing lifespan in nematode worms.[12]

Citric acid

Citric acid (2-hydroxy-propane-1,2,3-tricarboxylic acid) is a derivative of the Latin word citrus, the citrus tree, which be similar to a lemon fruit. Citric acid in its pure form is a tricarboxylic acid, colourless and freely water soluble having molecular weight of 210.14 Da. This acid is decomposable, sustainable, cost-effective, safe and a multipurpose chemical for wetting, buffering, cleaning, sequestering and dissolving. Citric acid is approximately most common transitional metabolic product and almost all animals and plants contain traces of this acid.[13]

A few bacteria for citric acid production are also suitable in addition to fungi and yeasts. By using mutation the fermentation competences of the selected strains can be improved.[14] The *Penicillium janthinellum* and the species of *Aspergillus* namely, *A. foetidus*, *A. awamori*, *A. wenti*, *A. carbonaries*, *A. fonsecaeus*, *A. phoenicis*[15] can also form citric acid in considerable volumes. Among yeasts, *Candida oleophils*, *C. guilliermondi*, S*accaromicopsis lipolytica*, *Hansenula anamola*, *Candida parapsilosis*, *Candida tropicalis*, *C. citroformans* and *Yarrowia lipolytica* can produce citric acid in considerable amounts. Among bacterial species, i.e. *Bacillus licheniformis*, *Arthrobacter paraffinens* and *Corynebacterium* sp. were also used previously for citric acid production by using

many raw materials as a substrate with the percentage yield of 27-88 % per sugar consumed by the microbial strains. However, in recent years, different strains of *Y. lipolytica* have been considered as the best citric acid producers.[13,16] Metabolism of the carbohydrate produces the basic unit of various aerobic and anaerobic microbes including citric acid, which is the key intermediate of carbohydrate metabolism. The microbial production of citric acid is considered to be purely through catalysis of enzymes. The each enzyme regulation synthesis (involved in the TCA cycle) is responsible for the production of Citric acid and the quantity of acid production depends upon enzymes regulation controlled by the cofactors and the metal ions which successively control trace element concentration for enzymes activity regulation (Figure 1) Shortly, it can be said that the overproduction of citric acid requires a distinctive combination of infrequent nutritional conditions (excess of hydrogen ions, carbon source, suboptimal concentrations of certain trace metals, dissolved phosphate and oxygen), which synergistically influence the fermentation performance. The deficiency of manganese, or phosphate and nitrogen limitation, inhibits the *A. niger* anabolism, and the resulting degradation of proteins leads to increased ammonium ion concentration. This increase is able to counterbalance the inhibition exerted by citric acid on phosphofructokinase, being a positive end-effector. High concentrations of NH_4^+ and glucose also repress the synthesis of α-ketoglutarate dehydrogenase, inhibiting the citric acid catabolism via the Krebs cycle, leading to its accumulation. Therefore, one of the reasons for the accumulation of citric acid is the result of high speed flow of income and a reduction in outflow velocity (Figure 2).[17]

Other smaller issues concerning citric acid production are of interest to other enzymes including: hexokinase, invertase, glucose oxidase phosphofructokinase, and other enzymes of the pyruvate kinase and pentose phosphate pathway.
The demand for the production of Citric acid by fermentation, is mounting frequently due to its wide range of applications. About 400,000 tons of citric acid are formed every year thorough fermentation process and the citric acid consumption per year is exceeding from 3.5–4.0 %.[18]

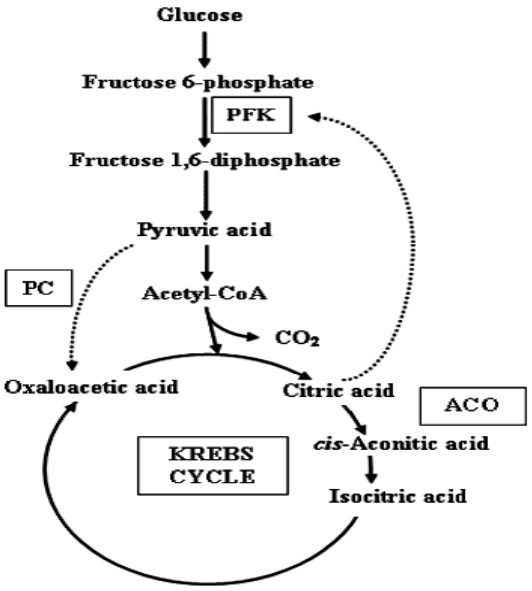

Figure 2- Diagrammatic image of the metabolic reactions in citric acid production by *A. niger.*[17] ACO = aconitase, PC = pyruvate carboxylase, PFK = phosphofructokinase.

Citric acid is a multipurpose and safe alimentary additive approved as GRAS (generally recognized as safe) by the Joint FAO/WHO Expert Committee on Food Additives. The pharmaceutical, cosmetics and food industries employ citric acid widely due to its universal recognition of safety, nice acid taste, high water solubility, buffering and chelating properties. Moreover, many complex molecules and commercially products are also formed by its carboxyl and hydroxyl groups.[18] Citric acid has also many uses in food and beverages industries such as; flavor inhancer, acidulant agent, antioxidant and preservatives. In agriculture and pharmaceutical industries citric acid has following uses; as fizzy in powders and pills mixed with bicarbonates, Micronutrient evaluation in fertilizers, anticoagulant make it available for vigorous dissolution of active ingredients, acidulant in mildly acerbic preparation.[17] Furthermore, few other industrial applications includes ectroplating, textiles, photographic reagents, copper plating, concrete, chemical conditioner on teeth surface, adhesives, plaster, polymers, floor cement, paper, tobacco, ion complexation in ceramic manufacture, waste treatment, and as well as in noncorrosive usage, non-hazardous and biodegradable processes that are according to the current ecological and safety standards.[13]

Fumaric acid

The fumaric acid which is also named as (*E*)-2-butenedioic acid or *trans*-1,2-ethylenedicarboxylic acid is naturally occurring organic acid and often also termed as "fumarates". It is a white solid with a low aqueous solubility. As compared to other carboxylic acids, it is relatively acidic.[19]

During the 1940s in the United States the production of fumaric acid through fermentation was exercised, but soon after, this procedure was stopped and restored by chemical synthesis from petrochemical feedstock, which give rise to the prices of petroleum again and develop the need for the production of fumaric acid by submerged fermentation.[20] While, the seldom use of *Rhizopus* species through fermentation fumaric acid production has been patented.[19] Moreover, Felix Ehrlich in 1911 discovered fumaric acid formation in *Rhizopus nigricans*, *Circinella*, *Mucor*, *Cunninghamella* and *Rhizopus* species.[21] While, fumaric acid as a minor fermentation product can be excreted by other microorganisms, such as *Saccharomyces cerevisiae* and some *Aspergillus niger* and *A. flavus* strains. The use of genetically engineered bacteria has been exercised as well for the production of fumaric acid with yields capability upto 85% w/w from glucose.[19]

In metabolic pathways such as the citrate cycle, fumarate is an intermediate between succinate and malate. In addition, fumarate plays a role in several biosynthetic and degradation pathways that will probably have no consequences for the fermentative yield of fumaric acid.[22] Fumaric acid biosynthesis takes place in both mitochondria and cytosol (Figure 3). Under aerobic conditions CO_2 fixation by pyruvate carboxylase catalysis illuminate the great molar yields of fumarate, which leads to oxaloacetic acid production, so that C_4 citrate cycle intermediates may be reserved for biosynthesis. When nitrogen is low and the growth phase is not working, glucose metabolism and CO_2 fixation might work continuously and results in accumulation of C_4 acids.[3]

For many potential industrial applications fumaric acid's carbon-carbon double bond and its two carboxylic acid groups make it appropriate. For esterification reactions and polymerization it can act as preliminary material.

As monomer for the production of polymers, such as; maleic anhydride, polyester resins usually unsaturated form, is currently favored more to use fumaric acid due to be inexpensive than fumaric acid. Nevertheless, fumaric acid could still be preferred, because of its non-toxic nature and because a more rigidity in the structure of polymer can be accomplished when fumaric acid is used. It is currently used in corn tortillas, wheat, sour dough, fruit juice, rye breads, refrigerated biscuit dough, nutraceutical drinks, gelatin desserts, pie fillings gelling aids, wine and as supplement in animal feed.[19] Fumaric acid increases value and decreases costs of most of food items and beverage products. It also increases feed efficiency for poultry and pigs. A very different application of fumaric acids is in a medicine to treat psoriasis.[23]

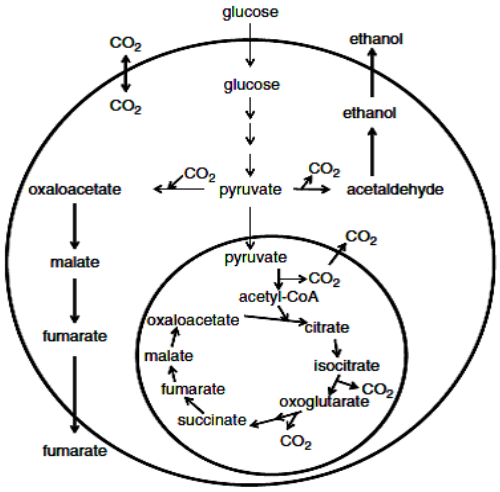

Figure 3- Main carbon flows during fumaric acid production. The *inner circle* represents the mitochondrial membrane, with citrate cycle reactions occurring in the mitochondria, while the *outer circle* represents the cytoplasmic membranes.[22]

Gluconic acid

The gluconicacid (GA) is a naturally occurring organic acid and most commonly known as polyhydroxycarboxylic acid found in macro and microorganism including humans. Gluconic acid is a nontoxic, soft, nonvolatile, mild organic acid which enables its extensive application in the pharmaceutical, food, leather and other industries. At neutral pH in aqueous solution, gluconic acid forms the gluconate ion. The "gluconates" are known to be the salts of gluconic acid.[24]

Gluconic acid can be produced by three different commercially methods i.e.; firstly, electrolytic addition of O_2 to glucose solution; secondly, chemical oxidation of glucose with a hypochlorite solution, comprising a known value of bromide and thirdly, fermentation process where definite microbes are cultivated in the production medium containing glucose as substrate and other ingredients.[25] Microbial fermentation process is very important striking techniques used to improve the problems regarding chemical

production for instance the predictable side reactions and also to further economize the bioprocess for the gluconic acid production.[27]

A varied group of microbes, including bacteria, yeast and particularly filamentous fungi have the ability to produce gluconic acid. In fungi, different species belonging to genera such as *Aspergillus, Gliocadium, Penicillium, Scopulariopsis* and *Gonatobotrys* have been experienced for the production of GA. While, many bacterial species, including *A. methanolicus, P. fluorescens, G. oxydans,* and the species of *Pseudomonas, Acetobacter*, *Morexella, Gluconobacter*, *Pullularia*, *Tetracoccus, Enterobacter* and *Scopulariopsis* participate in the production of GA with a particular pathway by oxidation of glucose with glucose dehydrogenase.[27] Furthermore, for commercial gluconic acid production several yeast strain such as; *A. pullulans* also offers a new prospect.[24,27]

Since billions of years from a diversity of raw carbohydrates the microbes have been found very well in producing primary and secondary metabolites. In current trend for GA fermentation, the study of enormous "Microbial Libraries" for microbes that have capability to alter the inexpensive carbohydrates into valueable end-products, that can provide as a raw material for the production gluconic acid.[24]

Fungal gluconic acid production is simple dehydrogenation reaction in which the conversion of glucose to gluconic acid occurs by catalysis of glucose oxidase enzyme (β-D-glucose: oxygen 1-oxidoreductase). Müller[28] first isolated this enzyme from a press juice got from *Penicillium glaucum*, which was formerly recognized as notatin. Glucose oxidase is a flavoprotein containing one very firmly but noncovalently bound FAD cofactor per monomer and is a homodimer having a molecular mass of 130–320 kDa depending on the amount of glycosylation. The glucose oxidase catalyzes the reaction, where glucose is converted to glucono-δ-lactone with removal of water molecule. While, hydrogen is moved to FAD. The consequential $FADH_2$ is restored to FAD by transfering of the hydrogen to oxygen to form hydrogen peroxide (Figure 4).

Due to growing concern in sustainable progress, there is wide call in the chemical, pharmaceutical and construction industries for the production of an absolute polyhydroxycarboxylic acid that has both reactive carboxyl and hydroxyl groups.[24] Thus, for almost 20 years the overall demand of this organic acid is growing with the time and currently its productional demand increases more than 87,000 tons per year and still rising.[29]

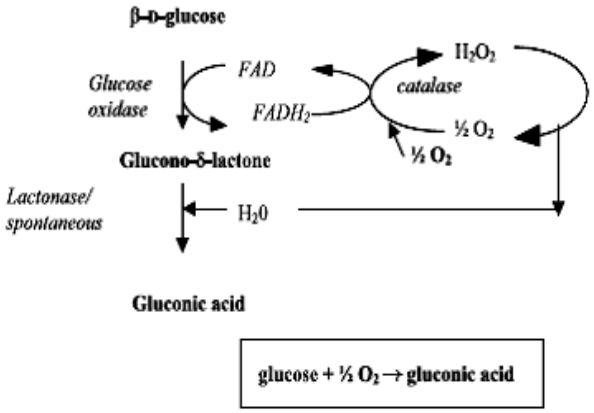

Figure 4- Oxidation of glucose by *Aspergillus niger*.[25]

The gluconic acid is a natural component of honey and fruit juices. Its inner ester, glucono-δ-lactone divulges firstly sweet taste which turns into somewhat acidic later. It has applications in dairy and meat products, mainly in baked items.[27] It also found some implementations in dropping fat absorption in cones and doughnuts.[24] It has multi potential and it was also used in food and concrete industries; as food additives, as bottle washing agent and retardant agent. Under extreme climatic conditions GA has been widely consumed in the construction industries and cleaning as an additive to enhance cement stability and resistance.[25]

Furthermore, different salts of gluconic acid have numerous applications; in term of a gel. Calcium gluconate is consumed to treat burns from hydrofluoric acid[30] and for more serious cases to elude necrosis of deep tissues calcium gluconate injections may be used; Sodium gluconate has the outstanding property to chelate calcium and other di- and trivalent metal ions; [31] Quinine gluconate, which is a salt between gluconic acid and quinine, which is consumed for intramuscular injection for the malarial disease treatment; Iron gluconate, injections have been proposed in the past to treat anemia[32] and also used in the textile industry due to its property of confiscating iron over a varied range of pH is oppressed, where it does not allow the addition of iron and for desizing polyester and polyamide fabrics and also prevents alkaline derusting;[24] Zinc gluconate, injections are used to neuter male dogs and to treat numerous diseases produced by zinc deficits such as skin changes, delayed sexual maturation and susceptibility to infections.[33]

Itaconic Acid

Itaconic acid (IA) which is a promising organic acid with the stoichiometric formula $C_5H_6O_4$ in which one carboxyl group is linked to the methylene group, which is unsaturated dicarbonic acid. It is a white crystalline acid and a molar weight of 130.1 g/mol.[34] The diversity of its functional groups make itaconic acid an effective intermediate in the production of complex organic compounds. The industrial versatility of itaconic acid and its reaction compounds is reflected in the broad range of applications.[35]

For some eras, the IA production metabolic pathway was under observations. Kinoshita in 1931 recommended a removal of CO_2 of aconitate as key reaction for IA production and discovered the IA production by *Aspergillus itaconicus*. After this discovery, microbial IA production becomes field of interest for researchers and further works start on it. Patents from the 1960's showed very good IA production processes.[36] But the results vary a lot, e.g., because of trace elements and/or reactor geometries. There are huge discrepancies with newer literature results, even with the same strains, fluctuate between shaking flasks, reactors and air-lift reactors.[34] So, reproducibility seems to be a major problem for IA production with this filamentous fungus. On the other hand, the demands for IA and its derivative production is still rising.

For IA production numerous kinds of microorganisms have been recognized in different studies including, *Candida* sp., *Ustilago zeae*, *Rhodotorula* sp.,[37] *Candida mutant*, *Aspergillus terreus* TN-484-M1 82 and *Aspergillus terreus* SKR10 20.[35] Up to now, *Aspergillus terreus* is the very commonly used as profitable IA producer. But, further studies to determine the factors influencing the process and to lower the production costs are necessary.

Many theories were proposed regarding the biosynthesis of itaconic acid using fungi, however Kinoshita (1932) stated that the main route of production is through glycolysis and tricarboxylic acid cycle.

During 1957, in cell extracts of *A. terreus* strong clues for the existence of a cis-aconitate decarboxylase (CAD) were shown by the studies of Bentley and Thiessen,[38] which speed up the reaction to IA, depicted in Figure 5. If started from glucose i.e. a glucose substrate, the carbon molecules are moved on through glycolysis to pyruvate. The pathway is then splitted and part of the carbon by releasing a carbon dioxide molecule is metabolized to acetyl-CoA while the other part is transformed to oxaloacetate so that the carbon dioxide molecule released earlier is again fused. In the early steps of the citric acid cycle *cis*-aconitate and citrate are produced while in the late step, which is special step producing only itaconic acid in which *cis*-aconitate decarboxylase (CadA) form itaconic acid liberating carbon dioxide. This pathway was proved with ^{13}C and ^{14}C labeled substrates by tracer experiments.[39-40]

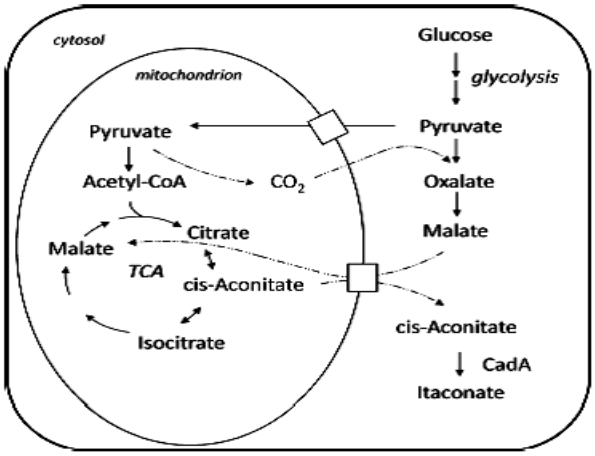

Figure 5- In the *A. terreus* between cytosol and mitochondrion cell biosynthesis pathway of itaconate and its compartmentalization.[41]

Itaconic acid made of renewable resources is gaining importance, due to its functionality; IA can be used for several specialties and so-called green plastics. Neither the preparation of polymers of IA nor the products of these plastics are health or environmentally damaging.[42] IA has been used extensively in industries with following applications; Improved adhesion to cellophane, deep shade in textile industry and well-organized dying, as a detergent, Industrial cleaner, dental fillers, Nonwoven fabric binder, as dental adhesives, thickeners in lubricating grease, Scale inhibitor in boiler. Furthermore, it also used as antifungal drug in ophthalmic drug delivery. Overall applications of IA make it much important for industrial scale production and china is one the four Itaconic acid biosynthesis countries in the world.[43]

Malic Acid

Malic acid is an organic acid belonging to C_4 dicarboxylic acids, which are structurally analogous to maleic anhydride and maleic acid, which characterize important building blocks in the chemical industry. In the future when increased gas and oil prices favor the formation of renewable chemicals from biomass, the C_4 dicarboxylic acids may therefore substitute petrochemically derived compounds. The salts

and esters of malic acid are known as malates. It has many applications in the beverage, food, agriculture, chemical and pharmaceuticals industries.

Although malic acid and calcium malate can be extracted from fruit and eggshells, these processes are not economical because fruit juices comprise less than 1.0% L-malate[44] and extraction from eggshells requires high cost, low extraction rates, a complicated operation, high energy-consumption and heavy pollution.[45] In fact, L-malic acid can be formed from pyruvic acid through oxaloacetic acid by one-step fermentation or is produced via the conversion of fumaric acid under catalysis of fumarase or is produced through acid hydrolysis of PMA.

There are three microbial groups which can synthesize L-malic acid, one of them through one-step fermentation produces L-malic acid from glucose, the other transforms fumaric acid to L-malic acid using pure fumarase or fumarase in its cells and the third group synthesizes PMA which can be hydrolyzed into L-malic acid.

Several wild and metabolically engineered strains were found to be able to produce L-malic acid. For example; *A.niger*,[46] *A. oryzae*, *A. flavus*,[47] *A. oryzae*,[48] *S. commune*,[49] *Penicillium sp.* K034,[50] *Z. rouxii*,[51] *A. pullulans* ZD-3,[52] *A. pullulans* ZX-10,[53] *P. rubens* 67, *P. viticola*152,[54] *Aureobasidium sp*. P6,[55] *S. cerevisiae*,[56] *A. oryzae* NRRL,[57] Engineered strains; *E. coli* WGS-10, *T. glabrata*, *E. coli* XZ658, *E. coli* XZ658,[58] and *B. subtilis*.[59]

For the synthesis of L-malic acid from glucose three metabolic pathways have been recognized. These include non-oxidative pathway, oxidative pathway and L-malic acid from the glyoxylate cycle.

The first pathway involves the Pyruvate carboxylation to oxaloacetate, followed by its reduction to malate, while *S. cerevisiae* lacks phosphoenolpyruvate carboxylase. If during glycolysis pyruvate is formed, this non-oxidative pathway is ATP neutral which contain a net fixation of CO_2, resulting in a highest theoretical malate yield of 2 mol (per mol of the consumed glucose). Many researchers consider that malate dehydrogenase, phosphoenolpyruvate (PEP) carboxylase and pyruvate carboxylase are involved in biosynthesis of malic acid in the non-oxidative pathway. Pyruvate carboxylase which is a biotin-dependent tetrameric enzyme and catalyzes the carboxylation of pyruvic acid, while malate dehydrogenase catalyzes the NAD(H)-dependent reversible conversion of L-malic acid to oxaloacetic acid, suggesting that NAD(H) and biotin are required during biosynthesis of malic acid.

While, the second pathway involves the Condensation of acetyl-coenzyme A (acetyl-CoA) and oxaloacetate to citric acid, followed by its oxidation to malate through the tricarboxylic acid (TCA) cycle, the conversion of glucose to malate through this oxidative pathway will result in the discharge of $2CO_2$. If acetyl-CoA is produced by pyruvate dehydrogenase, thus restraining the utmost theoretical malate yields to 1 mol (per mol glucose). In this case, fumarase which catalyzes the reversible water addition of fumaric acid to L-malic acid may play an important role in malate formation. Overexpression of either fumarase, which contain in the malate dehydrogenase or oxidative tricarboxylic acid (TCA) cycle in the reductive TCA cycle of mitochondria, raises the production of malic acid in *S. cerevisiae*.[60]

The third pathway involves the production of malate from two molecules of acetyl-CoA through glyoxylate cycle.[61] For malate production in this alternative oxidative pathway, the highest malate yield on glucose is restricted to 1 mol per mol due to the oxidative decarboxylation reaction essential for acetyl-CoA production from pyruvate. In the case, malate synthetase and iso-citrate lyase are involved in malate biosynthesis.[61]

Currently, the annual worldwide production of malic acid is 40,000 tones whereas the projected market volume is 200,000 tones.[62] The classical chemical procedure for malic acid production occurs at high pressure and increased temperature producing a racemic mixture of D- and L-malic acid and these current production costs are too high to allow a more wide spread use of L-malic acid.

As it possesses better taste retention and a greater acid taste than citric acid, malic acid as; flavor enhancer and an acidulant, is mainly consumed in beverages, food and candy. For example, crucial factors in defining the taste of Japanese sake are organic acids, such as lactic, Succinic and malic acids. The malic and succinic acids produced by the yeast *S. cerevisiae* during sake´ fermentation confer an umami and a refreshing taste to the sake.[63] Malic acid is also used as one component of antimicrobial agents. For example, the combination of Panax ginseng (2% v/v), malic acid (0.5% v/v) and potassium sorbate (0.05% v/v) show the maximum antimicrobial effectiveness against *Salmonella enteric* ser. Saintpaul and *E. coli* O157:H7 in sterile and fresh orange and mango juices, in addition to an elevated microbiological inhibition during storage (21 days).[64] L-Malic acid is also used in infusions of amino acid to treat liver dysfunction and hyper

ammonia. As the malic acid has been proposed as potential bulk chemical precursors, it may be used as a specialty chemical intermediate and a feedstock for chemical synthesis of biodegradable polymers, such as PMA as stated above. L-malic acid was also used for synthesis of optically pure 4-bromo-2-hydroxybutanoic acid esters which could be applied to synthesize peptide secondary structure mimetics.[65] L-Malic acid can be processed to provide ATP in the mammalian tricarboxylic acid cycle.

Calcium malate, ferric malate and zinc malate, formed during the fermentation, can be used as medicines to provide human and animals with calcium, iron and zinc. Malic acid is a naturally occurring organic acid in the plants and animals, including humans also known as fruit acid. In calcium malate the absorption potential of calcium is improved in competition to inorganic forms of calcium. Calcium malate also has application in ‘‘functional beverages’’. Knowledge of the requirement of calcium for long-term bone health has developed considerably and calcium fortification has shattered across the food chain. Phosphate consumption efficiency in soils is very low because in acidic soils applied phosphorus is mostly fixed to iron ($FePO_4$) and aluminum ($AlPO_4$) and to calcium [Ca_3 $(PO_4)_2$] in alkaline soils. Malic acid produced by *Penicillium oxalicum* may be mediated in aluminum phosphate solubilization in soil. For example, *P. oxalicum* demonstrated $AlPO_4$ solubilization than that of $FePO_4$ solubilization and higher levels of Ca_3 $(PO_4)_2$.[66] This means that malic acid may also be applied to agricultural industries.

Conclusion

The information from microbial genome sequences will speed up and simplify the identification of metabolic pathways, the collection of catabolic enzymes available to an organism, the uptake and export mechanisms, and regulatory genes, potential promoters etc. These developments in information, tools, and attitudes have the potential to accelerate the development of novel, efficient, economically feasible, and environmentally responsible fermentations for organic acid production. Taking these findings together it becomes evident that the quickest way to establish an industrial process for microbial organic acid production is the exploitation of natural producers with thorough bio-process engineering. Moreover, the choice of organism also influences by-product formation and therefore influences costs. Furthermore, a vigilant attention of the biodiversity of species is generally advisable – for the majority of small organic acids a natural producer might exist. The steps covered in this article are small aspects of microbial organic acid production as a whole, but taking them into account, the road leading to a sustainable society becomes more and more visible and thus, organic acids constitute a class of molecules with a great future ahead of them.

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